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The title of the invention has been amended (Guidelines for Examination in the EPO, A-III, 7.3).

(54) **Ethyl-(+)-apovincaminate for treating demyelinization clinical patterns of autoimmune origin.**

(57) The invention relates to a method for treating demyelinization clinical patterns of autoimmune origin, particularly the multiple sclerosis.

The invention further relates to a pharmaceutical composition useful for the treatment of demyenilization clinical patterns of autoimmune origin, particularly the multiple sclerosis.

The invention also relates to the use of ethyl (+)-apovincaminate for the preparation of a pharmaceutical composition useful for the treatment of demyelinization clinical patterns of autoimmune origin, particularly the multiple sclerosis.

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Description

METHOD AND PHARMACEUTICAL COMPOSITION FOR TREATING DEMYELINIZATION CLINICAL PATTERNS OF AUTOIMMUNE ORIGIN,

The invention relates to a method for treating demyelinization clinical patterns of autoimmune origin, particularly multiple sclerosis.

The invention further relates to a pharmaceutical composition which is useful for treating demyelinization clinical patterns of autoimmune origin, particularly multiple sclerosis.

The invention also relates to the use of ethyl (+)-apovincaminate for the preparation of a pharmaceutical composition which is useful for treating demyelinization clinical patterns of autoimmune origin, particularly multiple sclerosis.

It is known that, due to its action promoting the brain circulation and improving the oxygen utilization of cerebral tissues, ethyl(+)-apovincaminate (Cavinton®, vinpocetin) can therapeutically be used with success as a cerebral vasodilator (see e.g. British patent specification No. 1,405,127).

Based on results we have obtained in animal experiments our surprising conclusion is that ethyl (+)-apovincaminate can be used for treating demyelinization clinical patterns of autoimmune origin, particularly multiple sclerosis.

Ethyl (+)-apovincaminate can be prepared in a known way by nitrosating an octahydroindoloquinolizine derivative and treating the hydroxyimino-octahydroindoloquinolizine derivative obtained by an acid (British patent specification No. 2,102,415). Alternatively, ethyl (+)-apovincaminate can be prepared by using any process disclosed e.g. in DE Patent specifications Nos. 2,813,015 and 2,944,026; Japanese patent specification No. 1,237,552; British patent specification Nos. 2,036,744, 2,086,886 and 2,102,415; United States patent specification No. 4,400,514; as well as Hungarian patent specification No. 184,482.

Multiple sclerosis and other demyelinization clinical patterns of autoimmune origin such as e.g. perivenous encephalomyelitis (encephalomyelitis perivenosa) and acute haemorrhagic leukoencephalitis are diseases affecting the white substance of the human central nervous system. No therapeutic method is known up to the present which can be used for the successful treatment of this group of diseases.

Till now, the therapeutic efforts can be grouped in two categories: these are antiinflammatory-immunosuppressive therapies on the one part and supportive therapies for stabilizing the status of the patient on the other part. In the antiinflammatory therapy, steroids abolishing inflammation, cytostatics such as Cyclophosphamide or Azathioprine as well as the antibiotic Cyclosporin A as immunosuppressive agent and the combination of these drugs are usually employed. Similarly, other immunomodulatory treatment methods, aimed at influencing the effector phase of the immune response, e.g. treatment with α-interferon, whole-body irradiation (by X-rays) and the hyperbaric oxygen therapy may be used.

By "supportive therapy" we mean such therapeutic processes which are aimed to preserve the status of the patient. The sphere of these treatment methods is wide and includes e.g. vitamin cures (B_{12}, B_6), various physicotherapeutic methods and dietetic cures enriched in essential fatty acids.

Based on results obtained in animal experiments, we have found that ethyl (+)-apovincaminate is potentially useful for treating demyelinization clinical patterns of autoimmune origin, particularly the multiple sclerosis.

Accordingly, in one aspect, we provide use of ethyl (+)-apovincaminate in the preparation of a medicament for use in the treatment of demyelinization clinical patterns of autoimmune origin.

Ethyl (+)-apovincaminate may be useful either as such; or by using a pharmaceutical composition containing it together with any of the known agents used in the antiinflamatory-immunosuppressive therapeutic methods mentioned hereinbefore such as antiinflammatory steroids or cytostatics; or by using them in separate pharmaceutical compositions one after the other; or by using it together with any of the immunomodulatory treatment methods described hereinbefore or separately one after other; as well as by using it together with any of the supportive therapies as supplementation.

As a test model of the human demyelinization diseases, acute experimental allergic encephalomyelitis was chosen which is an artificially developed clinical status in animals, mainly in rodents, e.g. mice, rats or guinea-pigs [Neurochemicals Res. 6 (1981)]. Several methods are known for the evaluation of the symptons developed [J. Immunol. 132, 191 (1984)]; usually, the walking, defecation, histological alterations and changes of the immunological parameters of the animals are observed.

The investigations were carried out as described hereinafter.

50 µg of purified basic myelin protein and 100 µg of killed Mycobacterium tuberculosis were dissolved in 50 µl of sterile physiological saline solution buffered at pH 7 to 7.2 with disodium hydrogen phosphate and sodium dihydrogen phosphate and the solution was emulsified with 50 µl of Freund's complete adjuvant. The emulsion obtained was inoculated in the day 0 into the left posterior paw of inbred R9 and R9 albino guinea-pigs of both sexes, with 300 g of body-weight, which have been kept under standardized animal house conditions. Under the effect of the immunization, the animals got ill in the day 12 following the immunization and the level of deterioration in the group of the controls amounted to 90% in the day 14. The treatment was started simultaneously with the inoculation in the day 0. During the treatment groups of 3 to 5 animals were daily once intraperitoneally (i.p.) treated by ethyl (+)-apovincaminate dissolved in an ascorbic acid solution of 20% in daily doses of 0.25, 2.5 and 12.5 mg/kg, respectively. The survival of the animals was recorded as a most

complex measure of the drug therapy used which expresses the efficiency as well as harmful side effects of the therapy. The average survival time was determined by calculating the arithmetical mean value from the survival times of the individuals of the treated groups. The experiment lasted 21 days since a survival of 20 days is considered as a survival of the acute inflammation in the literature. The results are summarized in Table I.

Table I

Survival in days of guinea-pigs suffering from acute experimental allergic encephalomyelitis during an experimental period of 21 days

Treatment		Time of survival days
Substance	Dose mg/kg/day i.p.	
Control (physiological saline)		12.0
Ethyl (+)-apovincaminate	0.25	17.4
	2.50	20.4
	12.50	20.0

It is obvious from Table I that the animals were practically protected from the lethal outcome of acute experimental encephalomyelitis by using ethyl (+)-apovincaminate.

After the termination of the experimental period, the animals were killed by ether overdosage, dissected, then four regions of the central nervous system (frontally sectioned brain slices,brain stem-cerebellum, lower and upper segment of the spinal cord) were subjected to histological examination.

The histological examination is illustrated by Figures 1a, 1b, 1c and 2.

In the case of the control animals inflammation and accumulation of microglia cells were observed in the brain (Figure 1a), the brain stem (Figure 1b) and in the spinal cord (Figure 1c) with damage of neurons and demyelinization effects.

In sharp contradistinction, in the case of the animals treated with ethyl (+)-apovincaminate the inflammation was reduced substantially to a perivascular localisation due to which the walls of the blood vessels became thickened (Figure 2). There is no or very limited loss of neurons in the gray matter, resp. demyelinization in the white matter which is proved by Figure 2 showing a very limited number of inflammated cells and microglia cells, except the perivascular part, compared to the control.

More accurate determination of the numerical change and the ratio change of the inflamed cells and microglia cells, together with an explanation of the apparent histological changes could be the subject of further neuropathological examinations.

On the basis of the above facts it appears that ethyl (+)-apovincaminate extends the life span of animals suffering in acute experimental allergic encepha lomyelitis to a high degree, furthermore decreases the clinical differences and simultaneously the histological differences connected therewith. The inflammation of the central nervous system mostly did not damage the grey and white matter and appeared in the form of perivascular localisation in the animals treated effectively.

A comparative pharmacological study was also carried out by using Dexamethasone, a steroidal antiinflammatory drug employed for treating demyelinization clinical patterns of autoimmune origin, especially multiple sclerosis. This study was performed as described above, except that the treatment was started on outbred (and not inbred) guinea-pigs one day before the immunization and the daily doses were administered in two portions being possibly distant from each other, e.g. in the morning and in the afternoon. The solvent for Dexamethasone, i.e. physiological saline solution, and the solvent for ethyl (+)-apovincaminate, i.e., a tartaric acid solution of 0.75%, were used as controls. The treatment was carried out by using ethyl (+)-apovincaminate dissolved in tartaric acid solution of 0.75%, whereas a physiological saline solution of Dexamethasone was used as reference. The results of the treatments are summarized in Table II. In this Table,

the substances used for the treatments, the number of the animals treated and the time of appearance of the neurological symptoms are shown, which latter means the time, when the first neurological symptoms, usually ataxia, appeared on a given animal. These values were averaged for the animals receiving an identical treatment and the average value is shown in Table II. In the column "death", the average number of days from immunization up to the death of the animals is indicated. The number 21 means that no death occurred within the 21 days' study.

In the column of "survival", the percentage of treated animals surviving up to the end of the study, i.e. surviving for 21 days, is shown. The results obtained on the animals treated with ethyl (+)-apovincaminate were compared to the results obtained with a tartaric acid solution of 0.75%, being the solvent for ethyl (+)-apovincaminate, as control; the data of the animals treated with Dexamethasone were compared to the results obtained with physiological saline solution, being the solvent for Dexamethasone as control.

The difference between the time of appearance of the symptoms and the time of death was statistically insignificant in both control groups treated with either a 0.75% tartaric acid solution or physiological saline solution; thus, the course of the disease was practically identical in these groups.

Table II

Effect of ethyl (+)-apovincaminate on the acute experimental allergic encephalomyelitis of guinea-pigs

Treatment		No. of treated animals (pc.)	Appearance of symptoms (day)	Death (day)	Survival (%)
Substance	Dose (mg/kg/day i.p. in 2 portions)				
0.75% tartaric acid solution (control)		20	15.1	15.7	25
Ethyl (+)-apovincaminate	10.0	20	20.5	20.7	90
	12.5	15	20.6	20.8	93
	15.0	13	19.9	20.2	85
Physiological saline solution (control)		20	14.7	15.2	25
Dexamethasone (reference)	10.0	5	15.2	17.0	20
	15.0	5	17.8	19.6	60

It is obvious from Table II that, on the one hand, not more than 25% of 40 control animals survived whereas 85 to 93% (depending on the dose used) of the animals treated with ethyl (+)-apovincaminate survived, i.e. the decrease in the number of deaths was significant. On the other hand, when using ethyl (+)-apovincaminate in the given doses, the appearance of symptoms was significantly delayed; thus, the treatment can be considered to be successful, i.e. the symptoms did not appear even on the last day of the 21 days' treatment period, or, only in 2 cases between the day 19 and 20 of the treatment. Thus, the survival was nearly 100%. Conversely, in the case of treatment with Dexamethasone, a steroidal antiinflammatory drug most frequently used for treating this disease, the symptoms already appeared on day 15 of the treatment; the percentage of survival was not increased by using a 10 mg/kg dose; and the appearance of the symptoms was not significantly delayed but the death was inhibited by a 15 mg/kg dose, however, in the latter case the known side effects of steroids strongly manifested themselves.

A further important difference consists in that no toxic symptons occurred during the treatment with ethyl (+)-apovincaminate whereas on using Dexamethasone, the body-weight of the animals was enlarged by 60 to 70 g due to the water retention and they became unprotected against microorganisms. Thus, skin-mycosis

occurred on 2 out of 5 animals, 1 animal died due to peritonitis without any neurological symptom and an early stage of peritonitis was observed on 1 surviving animal during the dissection. Thus, the animals could be treated with ethyl (+)-apovincaminate for longer periods whereas, due to the immunosuppressive effect, lethal infections would be expected by using Dexamethasone.

The ethyl (+)-apovincaminate as active ingredient can be formed into pharmaceutical compositions useful e.g. for the treatment of multiple sclerosis by mixing it with known pharmaceutically acceptable, inert, non-toxic, solid enteral or parenteral administration. Such a procedure forms a further aspect of the invention. Suitable carriers are e.g. water, gelatine, glycerol, ethanol, lactose, cetyl alcohol, mannitol, silicic acid, carboxymethyl cellulose, alginates, polyvinylpyrrolidone, galactose, starch, pectin, magnesium stearate, stearic acid, sorbitol, kaolin, polyethylene glycol, fatty acid esters, talc, vegetable oils such as peanut oil or olive oil and the like. The active ingredient may be transformed to usual pharmaceutical compositions, e.g. solid forms (e.g. rounded or edged tablets, granulates, capsules such as hard gelatine capsules, pills, suppositories and the like) or liquid forms (e.g oily or aqueous solutions, suspensions, emulsions, syrups, soft gelatine capsules, injectable oily or aqueous solutions or suspensions and the like). The amount of the solid carrier may be varied within wide limits, preferably it weighes between 25 mg and 1 g. The compositions according to the invention may contain also commonly used pharmaceutical additives, e.g. preservatives, salts for adjusting the osmotic pressure, surfactants, buffers, dyeing, aromatizing and flavouring agents. Furthermore, the compositions may optionally contain other therapeutically active compounds which are suitable to treat demyelinization clinical patterns of autoimmune origin. The compositions are conveniently prepared in the form of dosage units corresponding to the desired route of administration, e.g. to enteral or parenteral (intramuscular, intraperitoneal, subcutaneous, intravenous particularly infusion, rectal and topical) use. These pharmaceutical compositions may be prepared by known methods, e.g. by sieving, mixing, granulating and compressing the components needed to the desired compositions. The compositions may be subjected to additional operations commonly used in the pharmaceutical industry such as coating the tablets, sterilization or the like.

The dose limits used of ethyl (+)-apovincaminate usually are between 0.05 and 50 mg/kg/day, optionally divided to two or more, preferably to two portions. The dose depends in each case on the patient, the severity of the disease, route of administration and the like.

The invention is illustrated in detail by the following non-limiting Examples.

Example 1

Preparation of tablets containing ethyl (+)-apovincaminate

Composition:

	mg
Ethyl (+)-apovincaminate	5.00
Colloidal silicic acid	1.25
Magnesium stearate	2.50
Talc	5.00
Starch	96.25
Lactose	140.00
	250.00

The above-defined amount of the active ingredient is mixed with the above-defined amounts of the additives, the mixture obtained is homogenized, granulated, subjected to drying by fluidization and then compressed to tablets each of which weighes 250 mg and contains 5 mg of the active ingredient.

Example 2

Preparation of an injectable solution containing ethyl (+)-apovincaminate

Composition:

	mg
Ethyl (+)-apovincaminate	10.00
Ascorbic acid	4.00
Sodium pyrosulfate	3.20
Tartaric acid	20.00
Benzyl alcohol	30.00
Propylene glycol	800.00

The above-defined amount of the active ingredient is mixed with the above-given additives and made up to 2 ml with distilled water. The solution is sterilized by filtration, filled into ampoules previously sterilized and sealed.

Claims

1. Use of ethyl (+)-apovincaminate in the preparation of a medicament for use in the treatment of demyelinization clinical patterns of autoimmune origin.
2. Use of ethyl (+)-apovincaminate according to claim 1 wherein the demyelinization clinical pattern is multiple sclerosis or allergic encephalomyelitis.
3. Use of ethyl (+)-apovincaminate according to claim 1 wherein the medicament is in the form of dosage units.
4. A process for the preparation of a pharmaceutical composition useful for the treatment of demyelinization clinical patterns of autoimmune origin characterized in that a therapeutically effective amount of ethyl (+)-apovincaminate and one or more other therapeutically active compound(s) commonly used for treating said disease are mixed together.
5. A process according to claim 4, wherein the one or more therapeutically active compound(s) are selected from anti-inflammatory agents, immunosuppressive agents and cytostatic agents.
6. A process according to claim 5, wherein the one or more therapeutically active compound(s) are selected from a steroidal compounds, cyclophosphamide, a zathioprine, cyclosporin A and α interferon.
7. A pharmaceutical composition comprising ethyl (+) -apovincaminate in admixture with one or more therapeutically active compounds.
8. A composition as claimed in claim 7, wherein the therapeutically active compound is selected from anti-inflammatory agents, immunosuppressive agents and cytostatic agents.
9. A composition as claim in claim 7 or claim 8 which further includes a pharmaceutically acceptable carrier, diluent or excipient.
10. A composition as claimed in any one of claims 7 to 9, wherein the therapeutically active compound is selected from steroids, cyclophosphamide, azathioprine, cyclosporin A and α-Interferon.

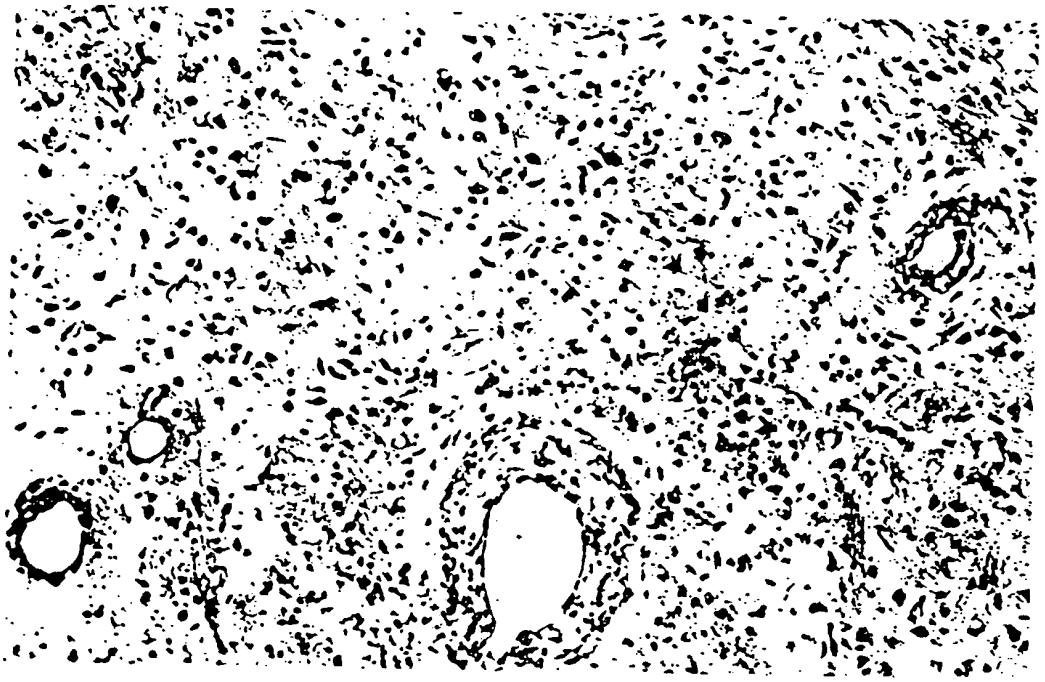

Fig. 1a

Fig. 1b